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Using particle tracking to probe the local dynamics of barley β-glucan solutions

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Abstract

The sol-gel transition of barley isolated β-glucan solutions which undergo gelation with ageing has been studied by conventional bulk rheology, phase contrast microscopy and particle tracking microrheology. Also, characterization of primary structure of the β-glucan isolate was carried out by HPLC. The Brownian diffusion of fluorescent microspheres (0.75 μm diameter, carboxylate-coated particles) was used to probe spatial mechanical properties of the gels at the scale of microns. The potential use of passive particle tracking as a new method of studying food systems that present spatial heterogeneities is explored. For the β-glucan gels cured at 25°C both the microrheology and the bulk rheology revealed that with increasing concentration of the polysaccharide the gelation time decreased, while the gelation rate and gel strength of the barley β-glucan gels increased. Moreover, the melting point increased with increasing concentration of the β-glucans indicating a better organization of the ordered domains in the network structure. The particle tracking method had higher sensitivity and could map molecular ordering and structural heterogeneities at a micro level. Furthermore, this method could detect changes in the structuring of the system before such events can be registered by the bulk rheological measurements. For these reasons, the timescales of the microscopic dynamic behavior do not always seem to match the timescales of the overall macroscopic behavior.

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Keywords: β-glucans; Particle tracking microrheology; gelation

1. Introduction

Cell walls of cereal grains, mostly barley and oat, are rich in mixed-linkage (1→3), (1→4)-β-D-glucans (β-glucans), which are linear polysaccharides of D-glucose residues interlinked via β-(1→3) and β-(1→4) linkages; their structure consists of consecutively (1→4)-linked β-D-glucose in blocks (i.e. oligomeric cellulose segments) that are separated by single (1→3)-linkages [1]. Cereal β-glucans display

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all the functional properties of viscous and gel forming food hydrocolloids combined with all the health benefits of soluble dietary fibers, such as reduction of plasma cholesterol and of postprandial serum glucose levels in humans and animals. These physical and physiological properties of cereal β-glucans largely depend on their molecular characteristics and polysaccharide concentration.

Passive particle tracking is a direct method consisting of observing the Brownian motion of tracer particles within a system and interpreting this movement in terms of the local viscoelasticity, or microrheology. In this type of passive microrheology, there is no external driving force applied to the tracer microspheres. The local particle motion is driven solely by Brownian forces generated by the thermal energy k_BT. Therefore, particle tracking can probe spatial variation in mechanical properties at the scale of microns without significantly distorting or altering the microstructure, providing that a sufficiently low concentration of tracer particles of appropriate size is employed. On the other hand, bulk rheological measurements describe the overall mechanical response of a material. That is, it is assumed that the sample is homogeneous and that there is no local variation in the structure. Although this assumption is valid for simple fluids, most colloidal systems are more complex and commonly present spatial heterogeneities [2, 3]. In order to understand the origins of the overall response, it is therefore necessary to probe rheology over shorter length scales and particle tracking provides this possibility.

2. Materials & Methods

In the present study, a barley β-glucan sample was used; this was an isolate from a barley concentrate supplied by CEBA (Lund, Sweden). The purification protocol for this preparation was described in detail by Lazaridou et al. (2003) [4] involving a dual-enzyme digestion with thermostable α-amylase and pancreatin followed by exhausting dialysis and precipitation of the polysaccharide by ethanol. The purity of the β-glucan isolate was estimated by determination of the barley β-glucan and protein contents using an (1→3, 1→4)-β-glucan assay kit purchased from Megazyme International Ltd (Bray, Ireland) and by the method of Lowry et al. (1951) [5], respectively.

The apparent peak molecular weight (Mw^p) of the β-glucan sample was obtained with a high performance size exclusion chromatography (HPSEC) system combined with a refractive index (RI) detector. Calculation of the Mw^p from the peak fraction of main eluting peak of the HPLC chromatogram was based on calibration with β-glucan standards isolated according to Lazaridou et al. (2004) [6] and having Mw^p of $466x10^3$, $300x10^3$, $186x10^3$, $83x10^3$, $33x10^3$ and $15x10^3$, as characterized by a light scattering technique [7]; eluting volumes of peak fractions for both standards and unknown samples were used in this calculation. The distribution of cellulosic oligomers in the chain of β-glucans was determined by treatment with lichenase [(1→3),(1→4)-β-glucan-4-glucanohydrolase, EC 3.2.1.73] and chromatography. High-performance anion-exchange chromatography (HPAEC), combined with a pulsed amperometric detector (PAD), was employed for analysis of oligosaccharides released from β-glucan by lichenase action. Description of the sample preparation and running conditions of both HPLC methods as well as of the determination of limiting viscosities [η] of the β-glucan sample using Ubbelodhe capillary viscometer are described in detail elsewhere [4].

The gel curing–melting events for the β-glucan preparations at different polysaccharide concentrations (2-5% w/w) were performed on a rotational Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) using double gap cylindrical geometry (bulk rheometry) as previously described [4].

Particle tracking: An optical microscope mounted on an Olympus BX61 microscope base, was operated in the fluorescence mode with a 100x oil-immersion objective of numerical aperture 1.25. The fluorescently labeled microspheres were added at a concentration of ~ 0.2 %v/v. The samples were then immediately placed into a welled slide filling it completely. Images were scanned approximately 10-20 μm below the level of the coverslip to minimize hydrodynamic interactions with the coverslip. All the measurements were conducted at 25°C. Trajectories of fluorescently labeled COOH-PS microspheres were recorded using an AVT Pike CCD camera at a frame rate of 30 Hz and short exposure time of

1000μs. Movies of the changing positions of the diffusing microspheres in the x–y plane were analyzed using the procedures of Crocker and Grier [8]. The number of simultaneously tracked microspheres per field of view was in the range of 80–100. For each of the particle tracking experiments reported here, approximately 1 minute of video (~1800 image frames) were recorded in digital format, yielding a few hundred thousand particle positions for each sample, and thus ensuring good statistical reliability. The trajectories are then used to calculate the ensemble-averaged mean-squared displacement (MSD) $\langle \Delta x^2(\tau) \rangle$.The MSD data obtained from particle tracking were converted accordingly to shear viscosity and to elastic and viscous moduli using a modified algebraic form of the generalized Stokes-Einstein equation. Detailed information on these analyses can be found elsewhere [9, 10].

3. Results & Discussion

3.1. Characterisation of b-glucan samples

The barley β-glucan preparation used in the present study was highly pure as evidenced by its low protein content (1.4% d.b.) and a high level of β-glucans (96.2% d.b.) (Table 1). The apparent Mw^p estimated from the HPSEC profile of the isolated polysaccharide was found 17 kDa, while the [η] value of the sample was 0.63 dl/g at 20°C. The characterization of primary structure of the β-glucan isolate was carried out by lichenase digestion, followed by HPLC quantification of the released oligosaccharides; this enzyme specifically cleaves the (1→4)-glycosidic bond of the 3-substituted glucose residues in β-glucans yielding oligomers with different degree of polymerization (DP). The major products for cereal β-glucans are 3-O-β-cellobiosyl-D-glucose (DP3) and 3-O-β-cellotriosyl-D-glucose (DP4), but cellodextrin-like oligosaccharides (DP ≥5) are also released from the polymer regions containing more than three consecutive 4-linked glucose residues. The calculated weight percent of the DP3, DP4 and cellulose-like oligosaccharides, DP5-9, for the lichenase digest of the β-glucan sample are given in Table 1. The calculated molar ratio of trimers / tetramers (DP3/DP4) was found around 3.5 for the barley β-glucan isolate used in this study. Both molecular weight and DP3/DP4 ratio in the cereal β-glucan chains have been identified as important determinants of the gelling capacity of these polysaccharides and the physical properties of the formed β-glucan gels [6].

Table 1. Compositional, molecular and structural features of the β-glucan isolate preparation

Compositional features		Molecular features		Structural features			
β-Glucans (% d.b.)	Protein (% d.b.)	$Mw^{p\,a}$ (x 10^3)	[η] (dl/g) (20°C)	$DP3^b$	$DP4^b$	$DP(5\text{-}9)^b$	Molar ratio DP3/DP4
96.20	1.42	17	0.63	69.21	26.30	4.49	3.48

3.2. Bulk rheological measurements.

The β-glucan preparation used in the present study exhibited structural features that promote aggregation phenomena among the polysaccharide chains, leading to gel structure formation, as it has a low Mw^p and a relatively high DP3/DP4 ratio [4, 6, 11]. The high gelation capacity of aqueous barley β-glucan dispersions, even at rather low concentrations (2-5 %w/w), was revealed by monitoring the storage (G'), and loss G´´ moduli, isothermally at different polymer concentrations using dynamic rheometry (Fig 1a). After an induction time, the storage modulus, G', increased with time and attained a plateau. The induction period and the gelation time (time at which G'=G´´, i.e. tanδ=1) decreased with increasing polysaccharide concentration (Fig 1a and Table 2). The maximum slope of the G´ curve was chosen as an

index of the gelation rate and was named as "elasticity increment, IE", and calculated as IE = (dlogG'/dt)max. As expected, the IE values increased with the polymer concentration (Table 2).

Table 2. Gelation kinetics of the β-glucan isolate preparation (frequency 1Hz, strain 0.1%, 25oC).

Concentration (%w/w)	Gelation time (min)	IE (h^{-1})	Melting temperature (^oC)
2	110.00 (±8.91)	1.34 (±0.15)	61.45 (±0.59)
3	64.83 (±5.34)	6.48 (±0.58)	63.33 (±0.65)
4	21.84 (±2.50)	10.60 (±1.12)	70.23 (±0.69)
5	12.31 (±0.95)	24.48 (±2.81)	75.15 (±0.78)

A drop in the G´ values is observed during heating of the β-glucan gels at a constant heating rate, implying melting of the network structure (Fig 1b). The melting point, defined as the temperature where G´ becomes equal to G´´ (i.e. tanδ=1), corresponds to the temperature at which chain dissociation in the junction zones occurs.

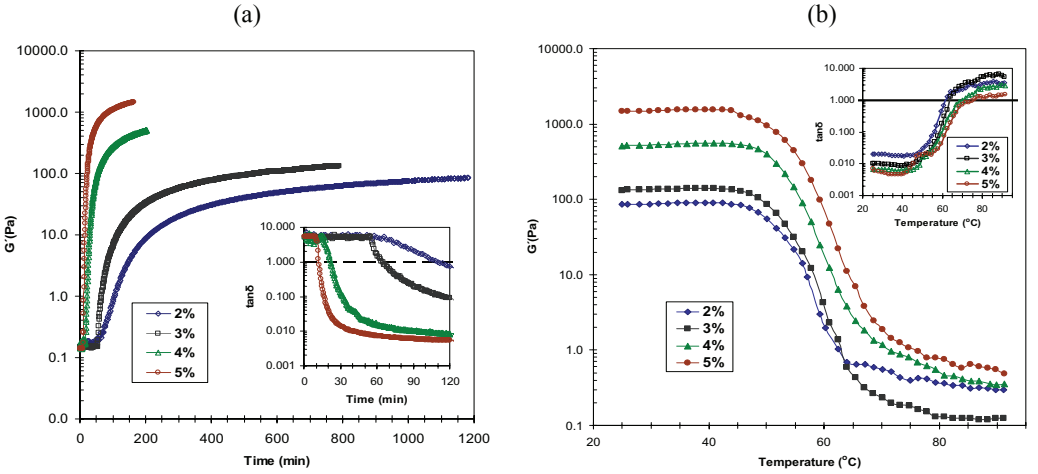

Fig. 1. (a) Gelation kinetics (G´, and tanδ -inset) for the barley β-glucan aqueous dispersions at different concentrations (%w/w) cured at 25°C (probed at 1Hz and 0.1% strain); (b) Temperature dependence of G´ and tanδ (inset) of gels cured at 25°C for the barley β-glucan isolate at different concentrations (%w/w); melting profiles were recorded at 1Hz, 0.1% strain, and 3°C/min heating rate.

The melting point of the barley β-glucan gels increased from 61.5 to 75.2°C with increasing the polysaccharide initial concentration from 2 to 5 % w/w (Fig. 1b, Table 2); a higher melting point shows a better organization of the ordered domains in the network structure. These results are in agreement with previous findings on the effect of cereal β-glucan concentration on the gelation properties of these polysaccharides [4, 11].

3.3. Particle tracking microrheology

The particle tracking results presented herein are only those for a typical concentration of β-glucan solution at 4% w/w; in fact similar behavior was observed for all β-glucan concentrations (2 to 5 % w/w) tested in this study. The structural heterogeneity is demonstrated by the distribution of displacements (p(Δx, τ)) [2]. For a homogeneous system, the set of particle displacements is described by a Gaussian fit.

A non-Gaussian distribution signifies that some of the probe particles experience different environments. The deviations from Gaussian behavior can be quantified using a non-Gaussian parameter N_G, defined as

$$N_G = (\langle \Delta x^4(\tau) \rangle / 3\langle \Delta x^2(\tau) \rangle^2) - 1 \quad (1)$$

which is zero for a Gaussian distribution (homogeneous system) and larger when the distribution is broader and deviates from Gaussian (heterogeneous system). Fig 2 shows the N_G parameter in a 4% w/w β-glucan solution, over all the lag times during ageing. It can be seen that the N_G increases with time. This means that as the network started to form, the particles became trapped inside pores in the aggregated β-glucan chain clusters that had different sizes and shapes. As the network formation advances, all the large clusters aggregate together and the final interconnected network (gel) is established. Beyond this point, the N_G increases further, the distribution of the displacements moves away from a typical Gaussian, indicating that the particles experience different environments even after the gel point. The N_G does not reach zero even at longer times suggesting local heterogeneities at a microscale level. Phase contrast microscopy (results not shown) revealed that clusters of aggregated β-glucans are generated during ageing. The size of the resulting pores also varied with the polysaccharide concentration: larger pores were observed in the gels made at lower biopolymer content.

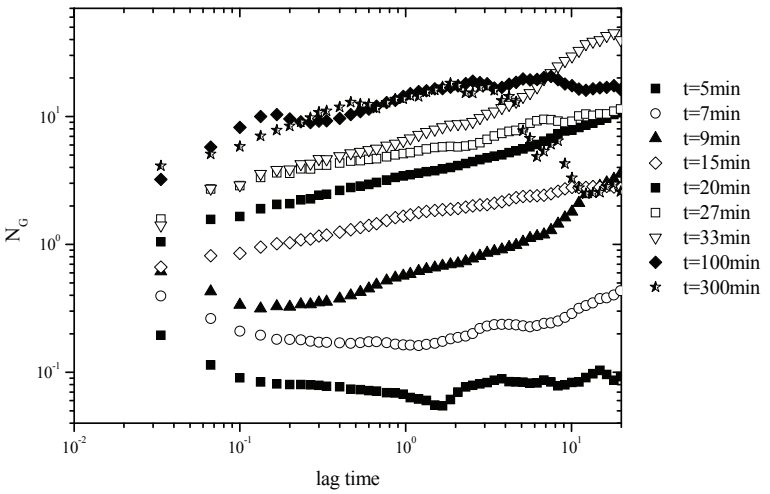

Fig 2. The non-Gaussian parameter N_G in 4% w/w β-glucan solution calculated from displacements over all lag times.

Fig 3 shows trajectories of particles (0.75 μm diameter, carboxylate-coated) from a typical time-series for a sample containing 4% β-glucan solution. When there is no significant viscoelastisity (t=5min), uniform trajectories are observed from freely diffusing particles. During ageing, the motion of the particles is constrained and the trajectories are less spatially extended. An elastic network is generated as the chain clusters jam together to fill the available space. As can be seen from Fig 3, the probe particles at t=21min, which is the gelation time obtained from bulk rheological measurements, experience different environments. That is, some trajectories are spatially extended and some others are dynamically arrested by the biopolymer network; i.e. some particles do move relatively large distances, while others become trapped inside the aggregated β-glucan network structure. As the intermolecular network formation further advances (t=100min), all the large clusters aggregate together diminishing the available aqueous space and smaller number of particles have extended trajectories. At even longer times (t=300min), the motion of all the embedded particles in the β-glucan network is constrained.

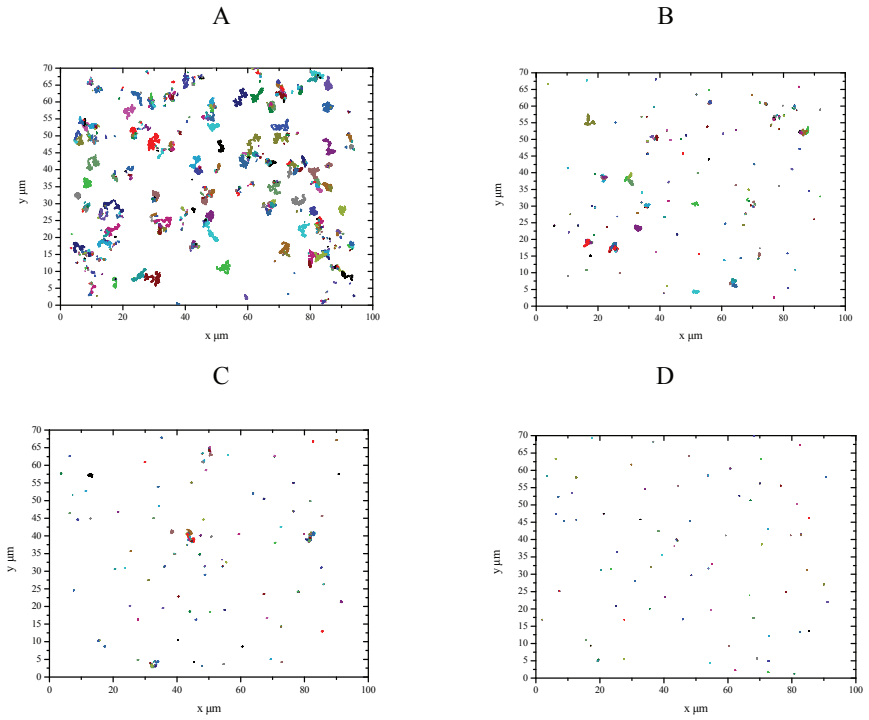

Fig 3. Trajectories of probe particles embedded in 4% w/w β-glucan solution at different times after sample preparation: (A) t=5min, (B) t=21 min, (C) t=100 min, (D) t=300 min

The ensemble-averaged mean-squared displacement $\langle \Delta x^2(\tau) \rangle$ (MSD) was calculated as a function of the lag time τ in order to quantify the dynamics of the probe particles. Fig 4 shows a typical time evolution of the MSD of probes in 4% β-glucan solution during ageing. Individual sets of data represent measurements taken for particles at different times after the preparation of the sample. At the beginning, when no gel network has been formed, the log-log plot had a slope of ~1 ($\langle \Delta x^2(\tau) \rangle \sim \tau$), indicating a purely viscous response of the surrounding medium. This initial viscosity can be determined by the Stokes-Einstein equation [9]. At this early time of gelation (t=5min), the value of $\eta = 0.01$ Pa.s. As the self-assembly occurs (i.e., the inter-molecular interactions become attractive), the slope of the log-log plot of MSD continuously decreases as the particles become progressively more constrained and trapped by the network. At longer times (t=300 min), the MSD becomes independent of the lag time, resembling the behavior of a purely elastic material.

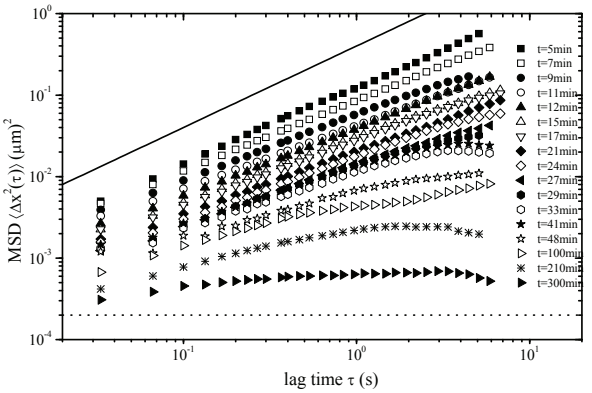

Fig 4. Ensemble-averaged mean-squared displacements (MSDs) versus lag time (τ) of carboxylate-modified microspheres (0.75 μm) in 4% w/w β-glucan solution during ageing; the solid line indicates the limiting value of slope = 1, while the dotted line indicates a slope = 0

4. Conclusion

We have demonstrated the potential of passive particle tracking microrheological studies in understanding the kinetics of β-glucan gelation. The method utilizes the forces generated by Brownian motion and as such the gelling system is distorted with the minimal strain possible and the polysaccharide aggregation kinetics can be monitored in situ. Phase contrast microscopy shows that clusters of aggregated β-glucan chains are generated during ageing. The sol–gel transition can be monitored via particle tracking, revealing that some probe particles experience different microenvironments during gelation; i.e. microheterogeneity in the evolving ordered polymer domains. The microrheological results were found to be in good agreement with the bulk rheological data. Furthermore, the particle tracking method could detect subtle changes in the developing network structure before such events can be registered by the bulk rheological measurements.

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